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Design, synthesis and the effect of 1,2,3-triazole sialylmimetic neoglycoconjugates on *Trypanosoma cruzi* and its cell surface *trans*-sialidase

Vanessa L. Campo [a], Renata Sesti-Costa [b], Zumira A. Carneiro [a], João S. Silva [b], Sergio Schenkman [c], Ivone Carvalho [a,*]

[a] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, Av. Café S/N, CEP 14040-903, Ribeirão Preto, SP, Brazil
[b] Faculdade de Medicina de Ribeirão Preto, USP, Av. Bandeirantes 3900, CEP 14049-900, Ribeirão Preto, SP, Brazil
[c] Departament of Microbiology, Immunology and Parasitology, Universidade Federal de São Paulo, Rua Botucatu 862, 8 Andar, 04023-062 São Paulo, SP, Brazil

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ABSTRACT

This work describes the synthesis of a series of sialylmimetic neoglycoconjugates represented by 1,4-disubstituted 1,2,3-triazole-sialic acid derivatives containing galactose modified at either C-1 or C-6 positions, glucose or gulose at C-3 position, and by the amino acid derivative 1,2,3-triazole fused threonine-3-*O*-galactose as potential TcTS inhibitors and anti-trypanosomal agents. This series was obtained by Cu(I)-catalysed azide–alkyne cycloaddition reaction ('click chemistry') between the azido-functionalized sugars 1-N_3-Gal (commercial), 6-N_3-Gal, 3-N_3-Glc and 3-N_3-Gul with the corresponding alkyne-based 2-propynyl-sialic acid, as well as by click chemistry reaction between the amino acid N_3-ThrOBn with 3-*O*-propynyl-GalOMe. The 1,2,3-triazole linked sialic acid-6-*O*-galactose and the sialic acid-galactopyranoside showed high *Trypanosoma cruzi trans*-sialidase (TcTS) inhibitory activity at 1.0 mM (approx. 90%), whilst only the former displayed relevant trypanocidal activity (IC_{50} 260 μM). These results highlight the 1,2,3-triazole linked sialic acid-6-*O*-galactose as a prototype for further design of new neoglycoconjugates against Chagas' disease.

1. Introduction

Trypanosoma cruzi trans-sialidase (TcTS) is a retaining glycosyltransferase that plays a key role in the pathogenesis of Chagas' disease—a parasitic blood-borne infection which affects many people in South and Central America.[1–3] The primary function of TcTS consists in the acquisition of sialic acid residues from mammalian host glycoconjugates and transfer of these monosaccharides to terminal β-Gal residues on the mucins that cover the parasite cell surface, generating α-2,3-sialylated-β-galactopyranose units.[4–6] Scavenging sialic acid from the host glycoconjugates aids the recognition and attachment of the parasite to host cells, and allows its evasion from the host's immune response, resulting in the host cells invasion where the parasite can complete its life cycle. TcTS is also shed from the parasite surface into the blood of the host and is able to act far from the infection site by inducing apoptosis in the spleen, thymus and peripheral ganglia.[7]

According to the determined crystal structure of TcTS by Buschiazzo et al. (2002),[8,9] the active site of this enzyme has several common features with microbial sialidases, being, however, composed by two distinct sites related to sialic acid (donor site) and to β-galactose molecule (acceptor site). The principal amino acids of the donor site are represented by an arginine triad (Arg35, Arg245 and Arg314) that interacts with the carboxylate group of sialic acid, besides other important residues essential for stabilization of the transition state (Tyr342, Glu230) and catalysis (Asp59). The acceptor site contains the amino acids Tyr119 and Trp312, which are crucial for the transglycosylation process, in addition to Asp59 (common to both sites) and Glu362 that directly interact with β-galactose acceptor.[8]

The most potent TcTS inhibitors described so far are represented by compounds that are able to occupy both donor (sialic acid) and acceptor (β-galactose) binding sites, so that the sialic acid transfer reaction is hindered or even blocked. As outlined in Figure 1, GM3 ganglioside **1**, when modified in its sialic acid residue, for instance at C-4 (deoxy or methoxy) or C-8 (deoxy), acted as TcTS inhibitor in a concentration range of 10–100 μM,[10] two sulfonamide chalcones **2** (IC_{50} 2.5 μM) and **3** (IC_{50} 0.9 μM), and the quinolinone **4** (IC_{50} 0.6 μM), obtained from chemical synthesis showed remarkable improvement against TcTS,[11] and a series of flavonoids and anthraquinones with also strong inhibitory activity against TcTS were identified from biological screening, being the lowest IC_{50} value observed for compound **5** (IC_{50} 0.58 μM).[12] More recently, 6-modified octyl-galactosides such as **6** and **7** showed strong TcTS inhibition (75% and 84%) in a radiochemical assay,[13] and the *C*-sialoside **8** obtained by cross metathesis displayed high affinity to TcTS (K_d 0.16 mM), as measured by surface plasmon resonance (SPR).[14]

* Corresponding author. Tel.: +55 16 36024709; fax: +55 16 36024879.
E-mail address: carronal@usp.br (I. Carvalho).

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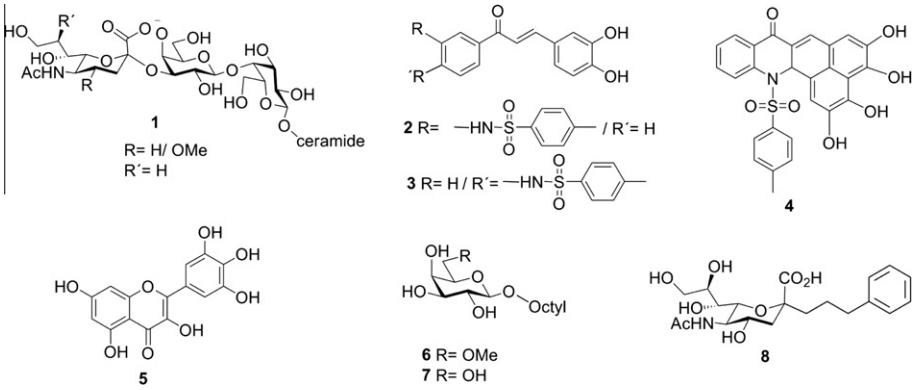


Figure 1. Representative TcTS inhibitors able to occupy both donor and acceptor sites of this enzyme.

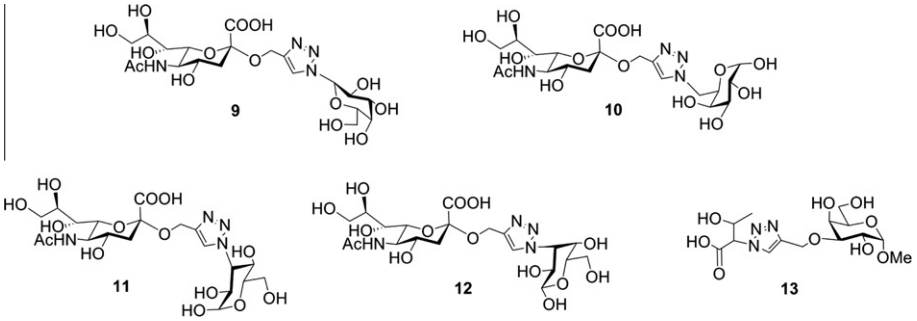


Figure 2. Structures of 1,2,3-triazole sialylmimetic neoglycoconjugates **9**–**13** as potential TcTS inhibitors.

Sialic acid derivatives and sialylmimetics have been largely utilized as scaffolds for the development of new drugs against various therapeutic targets.[15–28] In this context, Cu(I)-assisted 1,3-dipolar azide–alkyne cycloaddition (CuAAC) reactions have proved to be attractive to synthesize new sialylmimetics, considering that azides and alkynes precursors are relatively straightforward to introduce into organic molecules, besides being, generally, easily executed, fast and highly selective.[29,30] Indeed, they represent a valuable alternative strategy to overcome the main difficulties related to the synthesis of sialic acids glycosides, particularly the presence of carboxylate group at C-2, which may favors 2,3-elimination in glycosidation reactions, and the absence of C-3 substituent that impair the neighboring group participation to direct the stereochemical outcome of the formed glycoside.[15] Moreover, the physicochemical properties of the formed triazole group are particularly favourable, since it acts as a rigid link, displaying pharmacophore groups in well defined spatial orientation, and cannot be hydrolytically cleaved, oxidised or reduced.[31]

Therefore, we asked whether or not the mimicking of the terminal sugars α-D-Neu5Ac(2→3)-β-D-Gal of *T. cruzi* mucins by the 1,2,3-triazole sialic acid-based neoglycoconjugates **9**–**12** (Fig. 2), obtained by using CuAAC reactions, may contribute for strong and multivalent interactions at both donor and acceptor regions of TcTS active site. For purposes of further comparative enzymatic assays with TcTS the design of compounds **9**–**12** was envisaged based on the utilization of different azido-derived sugars (galactopyranose, glucopyranose or gulopyranose) along with an alkyne-functionalized sialic acid as precursors. Nevertheless, the fact that the negatively charged carboxylate group of sialic acid represent the most important group able to interact with sialic acid donor site through strong interactions with the arginine triad led us to also envision the design of relative simpler structures by replacement of sialic acid moiety by other containing carboxylate molecules like amino acids, as represented by the 1,2,3-triazole sialylmimetic **13** (Fig. 2). Thus, in this work we report the synthesis of the 1,2,3-triazole sialylmimetic neoglycoconjugates **9**–**13** and their biological evaluation as TcTS inhibitors and anti-trypanosomal agents.

In fact, the potential of compounds **9**–**13** to interact with essentials amino acids of both donor and acceptor sites was investigated by performing docking calculations into the TcTS active site.[†32] According to the results, all compounds were able to establish hydrogen bonding interactions with the arginine triad and Tyr342 at the donor site. Concerning interactions with the acceptor site, compounds **9**–**12** displayed hydrophobic 'π stacking' interactions between their triazole rings and Trp312, while galactosyl moiety of **13** was capable to interact, additionally, with both Trp312 and Tyr119 residues. The supra-cited interactions can be visualized in Figure 3, comprising compound **9**, representative of the designed 1,2,3-triazole sialic acid-based derivatives **9**–**12** and the sialylmimetic **13**. Based only on docking results it is possible to assert that sialic acid unit seems not to be a requisite for TcTS inhibition, considering that carboxylate group from threonine amino acid (compound **13**) was also able to interact with the donor active site, likewise sialic acid (compounds **9**–**12**). Furthermore, the different galactose, glucose and gulose sugar units of compounds **9**–**13** were capable to interact quite similarly with TcTS acceptor site, which means that galactose

[†] Previously to docking studies of compound **9**–**13** we performed docking between DANA and the donor active site of TcTS in order to validate the method with this enzyme. The good superposition between the DANA structure oriented with GOLD and the same molecule in the crystallographic orientation (PDB code 1MS8) suggested that the chosen method was appropriate.

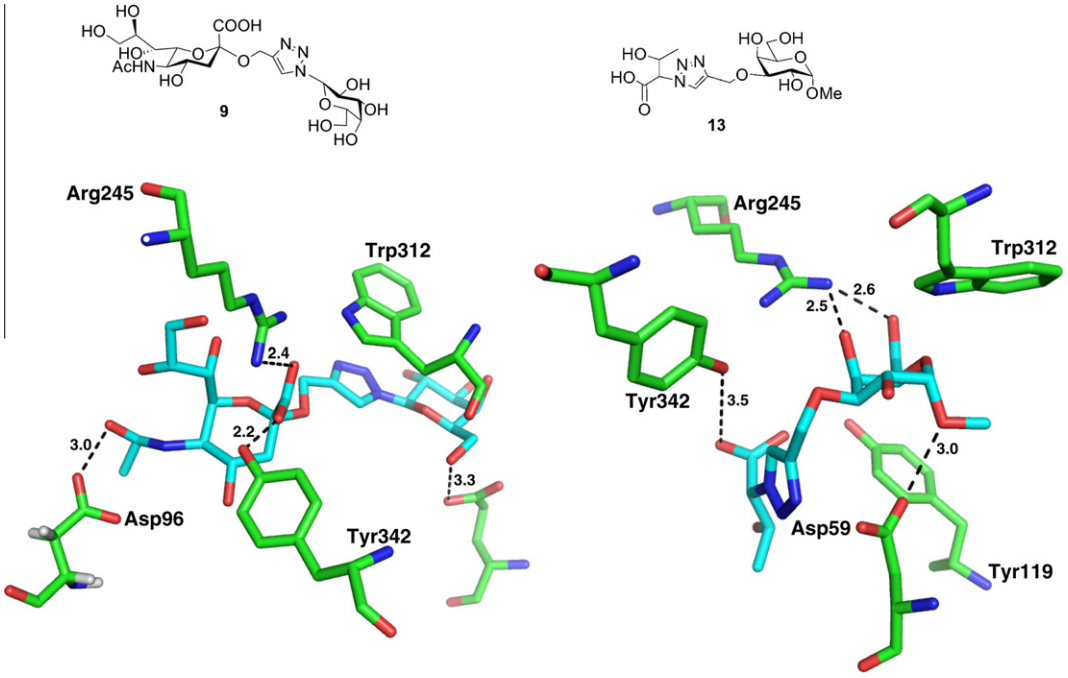


Figure 3. Docking results of compounds **9** (A) and **13** (B) in the TcTS active site.

does not constitute a requirement for design of TcTS inhibitors. Summing up, the number of significant interactions described for compounds **9–13** corroborate their capacity to interact with donor and acceptor sites, turning them, at least theoretically, potential TcTS inhibitors.

2. Results and discussion

2.1. Synthesis

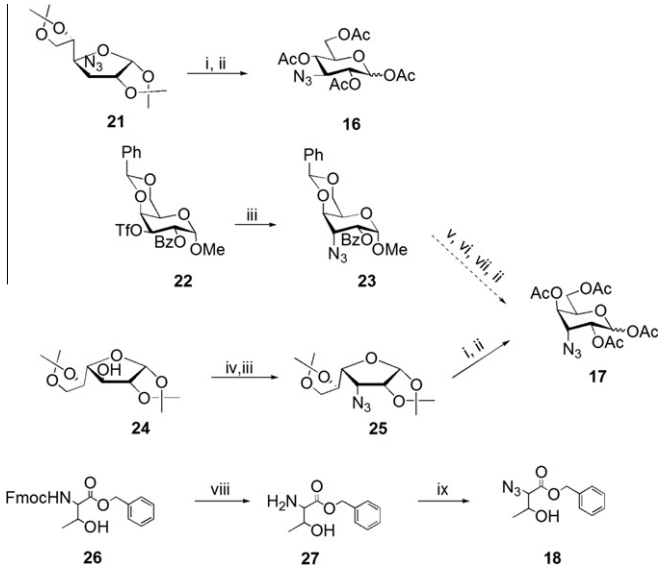
The target sialylmimetic neoglycoconjugates are represented by 1,4-disubstituted 1,2,3-triazole-sialic acid derivatives containing galactose modified at either C-1 (**9**) or C-6 positions (**10**), glucose (**11**) or gulose (**12**) at C-3 positions, and by the amino acid derivative 1,2,3-triazole fused threonine-3-*O*-galactose **13** (Fig. 2). The synthesis of compounds **9–13** by 'click chemistry' involved the use of azido-functionalized precursors represented by the sugars galactosyl azide **14** (commercial), 6-N_3-Gal **15**, 3-N_3-Glc **16** and 3-N_3-Gul **17**, and the amino acid N_3-ThrOBn **18**, as well as the utilization of the corresponding alkyne-based monomers 2-propynyl-sialic acid **19** and 3-*O*-propynyl-GalOMe **20**.

2.1.1. Synthesis of azido-functionalized sugars 15–17 and amino acid 18

The synthesis of 6-azido-functionalized galactopyranose **15** was performed in three steps, being the α isomer **15** isolated in 48% yield.[33–35] The synthesis of both 3-azido-functionalised glucopyranose **16** and gulopyranose **17** were carried out following the modified method described by Hindsgaul et al. (1994).[36a] Thus, starting with the synthesis of 3-azido-Glc **16**,[36a,b] the treatment of the allofuranose diacetonide **21**, obtained by sequential triflation/azide substitution reactions, with aqueous trifluoroacetic acid followed by per-acetylation gave compound **16** as a mixture of anomers (α/β 1:1) in total yield of 82% (Scheme 1). ^{1}H NMR analysis of **16** showed characteristic doublets of H-1 [α isomer (δ 6.25, $J_{1,2}$ 3.6 Hz); β isomer, δ 5.63, $J_{1,2}$ 8.2 Hz)] and shielded triplets relative to H-3 [α isomer (δ 3.93, *J* 10.3 Hz); β isomer (δ 3.66, *J* 10.3 Hz)], being the presence of azido group also confirmed by IR analysis [2104 cm^{-1} (N_3)].

Regarding the preparation of 3-azido-Gul **17**, different approaches were investigated, such as the azide substitution reaction of the intermediate methyl-2-*O*-benzoyl-3-*O*-triflate-4,6-*O*-benzylidene-α-D-Gal **22**,[37,38] which afforded the 3-azido-αGulOMe **23** with inverted C-3 configuration, albeit in low yield (33%) and still requiring additional laborious deprotections and acetylation steps (Scheme 1).[39] Alternatively, a synthetic approach to obtain the corresponding 3-azido-αGalOMe from the supra-cited intermediate **22** by means of double inversions at C-3 was not effective[40]; even though the subsequent reactions of **22** with $NaNO_2$ and Tf_2O furnished the corresponding 3-*O*-Tf-αGulOMe intermediate (76%), the following azide substitution did not give the desired 3-azido-αGalOMe.[38] To obtain the 3-azido-Gul **17** by a synthetic route involving fewer steps and in a better yield, Hindsgaul's method was followed as described for 3-azido-Glc **16**.[36a] Thus, triflation of galactofuranose diacetonide **24** followed by azide substitution reaction furnished the 3-azido **25** in 55% yield (Scheme 1), which was treated with aqueous trifluoroacetic acid and then per-O-acetylated to afford the final product **17** as an inseparable mixture of both anomers (α/β 0.5:1.0) in total yield of 80%. The structure of compound **17** was confirmed by IR [2112 cm^{-1} (N_3)] and NMR ^{1}H spectroscopy, which showed characteristic signals for H-1 [β isomer (δ 5.1, $J_{1,2}$ 8.2 Hz,); α isomer (δ 6.40, $J_{1,2}$ 3.0 Hz)], H-3 [β isomer (δ 5.08, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.4 Hz)], and $COCH_3$ (δ 2.19–1.99). HRESI-MS analysis of **17** showed the characteristic adduct $[M+Na]^+$ 396.10.

Concerning the preparation of the azido-functionalized N_3-L-ThrOBn **18**, the treatment of Fmoc-L-threonine benzyl ester **26** with 20% piperidine in DMF, followed by diazo transfer reaction of the resulting L-Thr-OBn **27** (78%), utilizing in situ generated triflyl azide and $CuSO_4$, gave the amino acid **18** (60%) (Scheme 1).[41,42,19] Its structure was confirmed by ^{1}H NMR [characteristic signals: OBn aromatic hydrogens (δ 7.39–7.33); βCHThr (δ 4.26) and αCHThr (δ 3.83)] and IR [2109 cm^{-1} (N_3)] spectra.

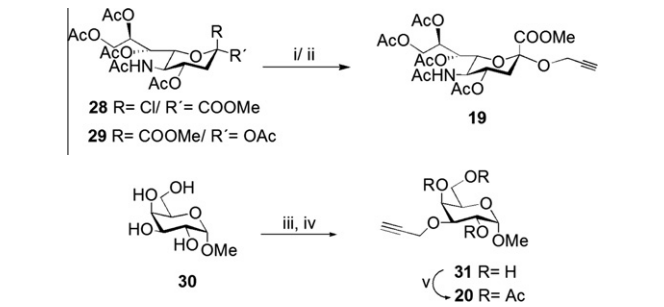


Scheme 1. Synthesis of the azido-functionalized sugars 3-N_3-Glc **16** and 3-N_3-Gul **17**, and the amino acid N_3-ThrOBn **18**. Reagents and conditions: (i) TFA 80%, rt; (ii) Ac_2O, Py, rt; (iii) NaN_3, 120–130 °C; (iv) Tf_2O, DCM–Py (19:1); (v) NaOMe, MeOH; (vi) AcOH 80%, microwave heating (110 °C, 15 min); (vii) Ph_3CBF_4, DCM, rt; (viii) 20% piperidine/DMF, rt; (ix) Tf_2O, NaN_3; $CuSO_4$, H_2O, MeOH, CH_2Cl_2, rt.

2.1.2. Synthesis of 2-propynyl-sialic acid 19 and 3-*O*-propynyl-GalOMe 20

The synthesis of 2-propynyl-sialic acid **19** was performed by glycosylation reaction of the chloride **28** with propargyl alcohol, in the presence of AgOTf as catalyst, being obtained in 66% yield after purification by column chromatography (EtOAc–Hexane 7:3) (Scheme 2).[43] Alternatively, $BF_3{\cdot}Et_2O$-catalysed glycosylation of the per-acetylated derivative **29** with propargyl alcohol also gave the desired product **19** in 70% yield.[44] The quite similar yields of **19** observed for both glycosylation procedures justify the employment of $BF_3{\cdot}Et_2O$-catalyzed reaction considering that it requires one less step. The presence of propargyl functional group in compound **19** was evident from characteristic signals of its ^{1}H NMR spectra [δ 2.43 ppm (CH) and δ 4.34, 4.02 ppm (CH_2)]. The α-anomeric configuration of **19** was confirmed with the aid of a 2D Heteronuclear Correlation Experiment (*G-BIRD$_{R,X}$-CPMG-HSQMBC*), being verified the value of 8.0 Hz for its $^3J_{C\text{-}1,H\text{-}3ax}$ heteronuclear coupling constant.[45,46]

The sugar 3-*O*-propynyl-GalOMe **20** was synthesized from commercial α-GalOMe **30**, by means of dibutylstannylene acetal formation with dibutyltin oxide, followed by in situ reaction with propargyl bromide.[47–50] Subsequently, per-O-acetylation of the obtained intermediate **31** (81%) under standard conditions provided the final product **20** (quantitative) after purification by column chromatography (EtOAc–Hexane 1:1) (Scheme 2). ^{1}H NMR analysis of **20** showed a doublet of H-1 (δ 4.96, $J_{1,2}$ 3.8 Hz), as well as characteristic signals of propargyl functional group [δ 2.44 ppm, *J* 2.3 Hz, (CH); δ 4.29 ppm, *J* 2.5 Hz, *J* 15.8 Hz, (CH_2)] and $COCH_3$ groups (δ 2.18–2.07).



Scheme 2. Synthesis of alkyne-based sugars 2-propynyl-sialic acid **19** and 3-*O*-propynyl-GalOMe **20**. Reagents and conditions: (i) propargyl alcohol, AgOTf, DCM, rt; (ii) propargyl alcohol, $BF_3{\cdot}Et_2O$, DCM, rt; (iii) Bu_2SnO, MeOH, rt; (iv) propargyl bromide, TBAI, toluene, rt; (v) Ac_2O, Py, rt.

2.1.3. Synthesis of potential TcTS inhibitors 9–13 by 'click chemistry' reactions

In general, the synthesis of compounds **9–13** by Cu(I)-assisted 1,3-dipolar azide–alkyne cycloaddition reactions were performed in a microwave reactor utilizing the catalytic system $CuSO_4$/sodium ascorbate and DMF as solvent.[51,52] The progress of the reactions was followed by TLC analysis, which revealed complete consumption of starting materials and no further changes after 15 min irradiation bursts (3) at 100 °C. Thus, the condensation of sugars **14**, **15**, **16** and **17** with 2-propynyl-sialic acid **19** afforded the per-acetylated products **32** (32%), **33** (34%), **34** (74%) and **35** (43.4%) after purification by column chromatography (EtOAc), being compounds **34** and **35** isolated as single α and β anomers, respectively (Scheme 3). The structures of **32–35** were confirmed by ^{1}H NMR analysis, which showed a characteristic singlet of C*H*-triazole around δ 8.0, singlets relative to $COCH_3$ and $NHCOCH_3$ groups between δ 2.2 and 1.9, with integration value of 27, besides other characteristic signals of sugars and sialic acid units. Other relevant evidence was the absence of the propargyl-CH signal at δ 2.43 ppm. As described for compound **19**, $^3J_{C\text{-}1,H\text{-}3ax}$ heteronuclear coupling constant values in the range of 8.0–8.7 Hz were verified for sialosides **32–35**, confirming their α-anomeric configuration.[45,46] HRESI-MS analysis showed the characteristic adducts of $[M+Na]^+$ 925.28 for compounds **32**, **34** and **35**, and $[M+H]^+$ 903.29 for compound **33**.

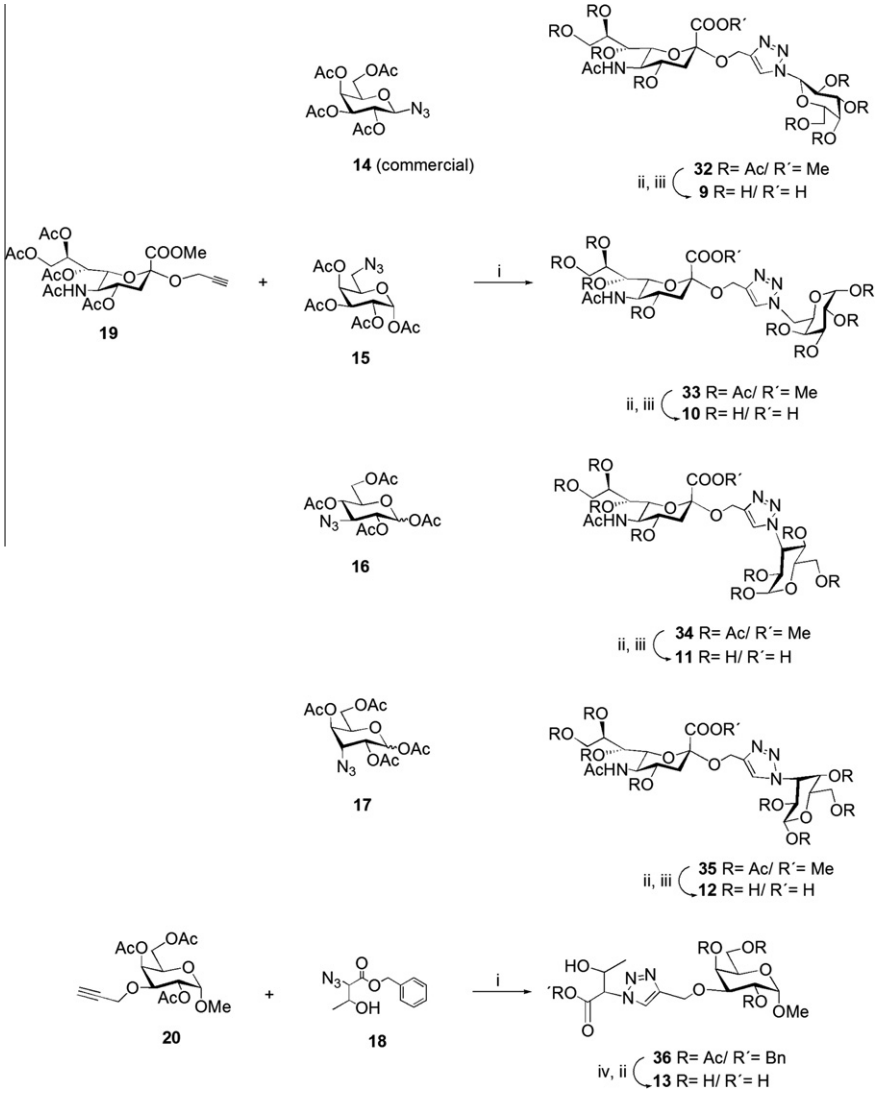
Compounds **32–35** were then deacetylated by treatment with 1 M NaOMe, followed by de-methyl-esterification using 0.2 M KOH (Scheme 3).[27] The resulting materials were purified using reverse-phase HPLC, affording the corresponding deprotected products **9–12** in low to moderate yields (13–47%), considering that their purification by HPLC was not straightforward, as it required the use of several experimental conditions. The structures of compounds **9–12** were confirmed by ^{1}H NMR spectroscopy, which showed absence of singlets relative to CO_2CH_3 and $COCH_3$ groups, being verified only one singlet of $NHCOCH_3$ at δ 1.9 ppm.

Regarding the synthesis of compound **13**, click reaction between N_3-L-ThrOBn **18** and 3-*O*-propynyl-GalOMe **20** gave the per-acetylated product **36** in 48% yield after purification by column chromatography (EtOAc–Hexane 7:3) (Scheme 3). Its ^{1}H NMR analysis showed characteristic signals of sugar ($OCOCH_3$, δ 2.11–2.06) and amino acid (OBn, δ 5.31–5.22), as well as CH-triazole (δ 7.97) and CH_2-triazole (δ 4.69–4.4; *J* 12.4 Hz) signals. Subsequently, removal of the benzyl ester from **36** by means of standard hydrogenation (10% Pd–C/H_2) and deacetylation reaction in the presence of 1 M NaOMe in MeOH afforded the final deprotected product **13** in 80% yield.

2.2. Biological assays

2.2.1. 1,2,3-Triazole sialylmimetic neoglycoconjugates 9–13 as inhibitors of *Trypanosoma cruzi trans*-sialidase

The enzymatic inhibition assay involving compounds **9–13** was performed using the continuous fluorimetric method, which is based on TcTS-catalyzed hydrolysis of MuNANA.[53] Compounds **9–13** were tested at 1.0 and 0.5 mM concentrations, along with DANA as control, which is reported to be a weak TcTS inhibitor.[54] According to the obtained results (Fig. 4), compounds **9** (88%), **10** (91%), **11** (67%) and **12** (69%) showed high inhibition of TcTS at



Scheme 3. Synthesis of 1,2,3-triazole-linked sialic acid and amino acid glycosides **9**–**13** by 'click chemistry'. Reagents and conditions: (i) $CuSO_4$, Na ascorbate, DMF, microwave heating (100 °C, 15 min); (ii) NaOMe, MeOH; (iii) KOH 0.2 M; (iv) H_2/10% Pd/C.

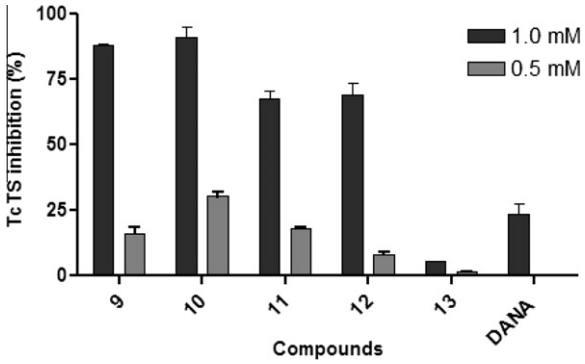


Figure 4. TcTS inhibition of compounds **9**–**13**.

1.0 mM, whereas almost no inhibitory activity was detected for compound **13** (5%) at this concentration. However, when tested at 0.5 mM concentration, only compound **10** (30%) displayed a moderate inhibition of TcTS, being weak inhibition values (1.5–17%) verified for the remaining compounds. The effective inhibition of TcTS by compounds **9**–**12** at 1.0 mM are in accordance with docking results (Section 1), despite the fact that compounds **9** and **10**, containing galactosyl units, exhibited higher activity against TcTS than compounds **11** and **12**, constituted by corresponding glucose and gulose sugar units. On the other hand, the very low inhibition displayed by compound **13** (1.0 mM), obtained from threonine amino acid in replacement of sialic acid, is not in agreement with its docking result, indicating that sialic acid unit may have a relevant role in TcTS inhibition. Therefore, the significant TcTS inhibition showed by compounds **9**–**12**, albeit only at 1.0 mM, may direct the development of new sialylmimetics as TcTS inhibitors, pointing out the necessity to maintain sialic acid and galactosyl units for efficient inhibition of this enzyme. Lastly, the increasing repertoire of biological activities described for sialylmimetics opens up the possibility to test compounds **9**–**13** against other essential therapeutic targets, such as neuraminidase.[15,16]

2.2.2. In vitro trypanocidal activities of compounds 9–13 and cytotoxicity towards mammalian cells

The five 1,2,3-triazole sialylmimetic neoglycoconjugates **9–13** were evaluated against trypomastigote forms of *T. cruzi* Tulahuen

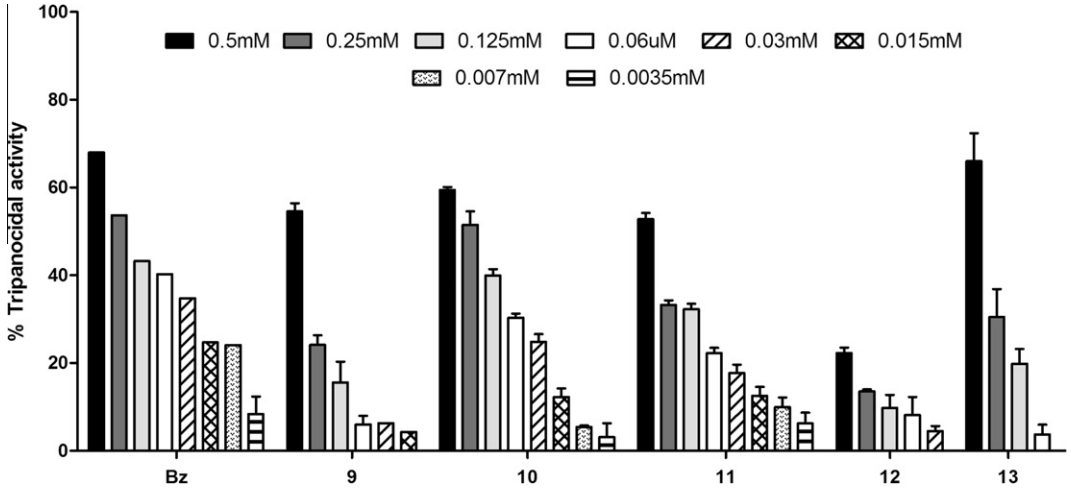


Figure 5. Trypanocidal activities of compounds **9**–**13** and reference compound benznidazole (Bz).

strain using benznidazole (*N*-benzyl-2-nitro-1-imidazolacetamide), the current frontline drug used to treat Chagas' disease, as control.[55]

Results of parasite viability, measured based on a colorimetric reaction with Chlorophenol red-β-D-galactoside (CPRG) as substrate for *T. cruzi* β-galactosidase, are summarised in Figure 5.[56] The concentrations of compounds corresponding to 50% trypanocidal activity are expressed as IC_{50try} (Table 1). As shown in Figure 5 and Table 1, amongst the tested compounds **10** displayed higher trypanocidal activity, presenting an IC_{50try} value of 260 μM against *T. cruzi* Tulahuen strain. In fact, this compound presented trypanocidal activity comparable to the reference benznidazole (Bz, IC_{50try} 125 μM) in the concentrations range from 0.5 to 0.06 mM. Regarding the remaining compounds, overall relative low activity (**9** and **11**–**13**) was observed, despite the high trypanocidal activity presented by **9**, **11** and **13** at 0.5 mM concentration.

Table 1
Trypanocidal activities of compounds **9**–**13** and benznidazole (Bz) expressed as IC_{50Try}

Compound	IC_{50try} (mM)
9	0.475 ± 0.054
10	0.26 ± 0.018
11	0.726 ± 0.073
12	>2
13	0.369 ± 0.076
Bz	0.125 ± 0.034

Concerning the mammalian cell toxicity, compounds **9–13** were screened against cultured mouse spleen cells. According to Figure 6, high levels of cytotoxicity were verified only for compounds **9** and **13** (above 80%) at 0.5 mM, followed by compound **10** that showed around 55% mouse cell kill at this increased concentration, being, however, not cytotoxic in lower concentrations. For the remaining compounds **11** and **12** no cytotoxicity was observed in the 500–7.8 μM range.

Although TcTS is essential to enabling parasite evasion of the human immune response, adhesion to and invasion of host cells, it is not known to play a role in parasite survival in culture.[51] In this context, the obtained TcTS inhibition and anti-trypanosomal activities were not directly correlated, suggesting that the tested compounds may act against *T. cruzi* by a different mechanism of action. Furthermore, considering that significant cytotoxicity against mouse spleen cells was observed only at high concentrations of the tested compounds, a specific mode of anti-parasite action rather than a generic cytotoxic effect may be suggested.

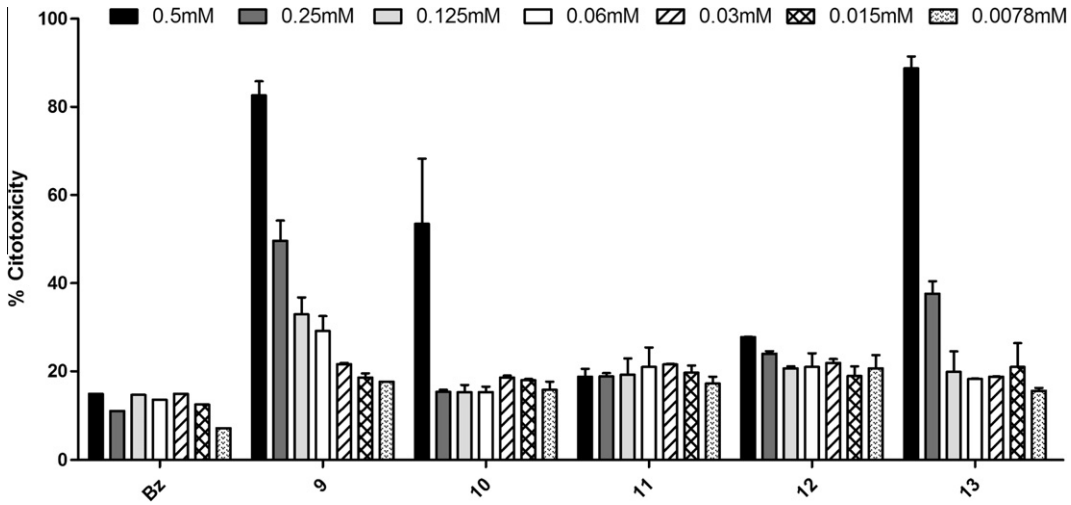


Figure 6. Percentage cell death caused by compounds **9**–**13** and Bz, evaluated against cultured mouse spleen cells.

3. Conclusions

In summary, we have developed the synthesis of five new 1,2,3-triazole sialylmimetic neoglycoconjugates from different azido-derived sugars (Gal, Glc, Gul)/amino acid (Thr) and alkyne-functionalized sialic acid/galactose as precursors. The use of Cu(I)-catalysed azide–alkyne cycloaddition reaction proved to be effective for generating the more resistant 1,2,3-triazole rings as mimics of natural *O*-glycosidic linkages, thus representing a valuable strategy to get a repertoire of neoglycoconjugates.

Trypanosoma cruzi trans-sialidase (TcTS) inhibition assays with compounds **9–13** provided evidence that sialic acid and galactosyl units are relevant for TcTS inhibition, due to higher inhibitory activities verified for **9** and **10** at 1.0 mM, whilst trypanocidal assays showed higher anti-parasite activity for compound **10** (IC_{50try} 260 μM). Taken together, these data highlights the 1,2,3-triazole linked sialic acid-6-*O*-galactose **10** as a prototype for the development of more effective candidates against Chagas' disease.

4. Experimental

4.1. General

All chemicals were purchased as reagent grade and used without further purification. Solvents were dried according to standard methods.[57] MuNANA (2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid sodium salt), used as a donor substrate for silylation reactions, was acquired from Toronto Research Chemicals Inc. The *trans*-sialidase used in this study was a His-tagged 70 kDa recombinant material truncated to remove *C*-terminal repeats but retaining the catalytic *N*-terminal domain of the enzyme.[58] Reactions were monitored by thin layer chromatography (TLC) on 0.25 nm precoated silica gel plates (Whatman, AL SIL G/UV, aluminium backing) with the indicated eluents. Compounds were visualized under UV light (254 nm) and/or dipping in ethanol–sulfuric acid (95:5, v/v), followed by heating the plate for a few minutes. Column chromatography was performed on Silica Gel 60 (Fluorochem, 35–70 mesh) or on a Biotage Horizon High-Performance FLASH Chromatography system using 12 or 25 mm flash cartridges with the eluents indicated. The microwave-assisted reactions were carried out in a laboratorial oven (CEM DISCOVER) using sealed tubes. HPLC purifications were performed in the Shimadzu HPLC system using a Shim-PaK CLC-ODS (M) semipreparative reverse phase column (250 × 10.0 mm). Nuclear magnetic resonance spectra were recorded on Bruker Advance DRX 300 (300 MHz), DPX 400 (400 MHz) or DPX 500 (500 MHz) spectrometers. Chemical shifts (δ) are given in parts per million downfield from tetramethylsilane. Assignments were made with the aid of HMQC and COSY experiments. Optical rotations were measured at ambient temperature on a Jasco DIP-370 digital polarimeter using a sodium lamp. Accurate mass electrospray ionization mass spectra (ESI-HRMS) were obtained using positive ionization mode on a Bruker Daltonics UltrOTOF-Q-ESI-TOF mass spectrometer.

4.2. Synthesis

4.2.1. 1,2,4,6-Tetra-*O*-acetyl-3-azido-3-deoxy-α,β-D-glucopyranose 16[36a,b]

A solution of azido **21** (108 mg, 0.41 mmol) in 80% aqueous TFA (1 mL) was stirred at room temperature for 1 h. The mixture was then evaporated in vacuo and to the resulting oil were added pyridine (3 mL) and acetic anhydride (2 mL). After stirring for 12 h at room temperature, the solvents were removed under reduced pressure and the residue was diluted with DCM. The solution was washed with 1 M HCl, and satd aq $NaHCO_3$ solution, dried over $MgSO_4$, concentrated under reduced pressure and purified by column chromatography (EtOAc–hexane 1:1) to give product **16** (126 mg, 0.34 mmol, 82%) as a mixture of anomers (α:β 1:1). δ_H ($CDCl_3$, 300 MHz). α anomer: 6.25 (1H, d, $J_{1,2}$ 3.6 Hz, H-1), 4.98 (1H, t, *J* 10.14 Hz, H-4), 4.90 (1H, dd, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.6 Hz, H-2), 4.17 (1H, dd, $J_{5,6}$ 4.6 Hz, $J_{6,6'}$ 12.0 Hz, H-6), 4.05 (1H, m, H-6′), 3.93 (1H, t, *J* 10.29 Hz, H-3), 3.75 (1H, ddd, $J_{5,6'}$ 2.3 Hz, $J_{5,6}$ 4.6 Hz, $J_{4,5}$ 9.8 Hz, H-5), 2.15–2.03 (12H, 4 s, $COCH_3$). δ_C ($CDCl_3$, 100 MHz) 171.0, 169.7, 168.6 ($COCH_3$), 87.8 (C-1), 73.2 (C-5), 69.9 (C-2), 69.8 (C-4), 61.5 (C-6), 60.5 (C-3), 21.1–20.8 ($COCH_3$).

β anomer: 5.63 (1H, d, $J_{1,2}$ 8.2 Hz, H-1), 4.99 (1H, dd, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 10.14 Hz, H-2), 4.18 (1H, dd, $J_{5,6}$ 4.6 Hz, $J_{6,6'}$ 12.0 Hz, H-6), 4.04–3.96 (3H, m, H-6′, H-4, H-5), 3.66 (1H, t, J 10.14 Hz, H-3), 2.15–2.03 (12H, 4 s, $COCH_3$). δ_C ($CDCl_3$, 100 MHz) 171.0, 169.7, 168.6 ($COCH_3$), 91.2 (C-1), 69.5 (C-5), 67.3 (C-2), 69.7 (C-4), 64.3 (C-3), 61.5 (C-6), 21.1–20.8 ($COCH_3$).

IR (KBr) ν_{max} 2961, 2104, 1759, 1372, 1234, 1040 cm^{-1}.

ESI-HRMS: calcd for $C_{14}H_{19}N_3O_9Na$ $[M+Na]^+$: 396.1121, found: 396.1008.

4.2.2. 3-Azido-3-deoxy-1:2,5:6-di-*O*-isopropylidene-α-D-gulofuranose 25

To a solution of 1:2,5:6-di-*O*-isopropylidene-α-D-galactofuranose **24** (280 mg, 1.07 mmol), in anhydrous DCM (10 mL) and pyridine (0.5 mL) at 0 °C was added dropwise triflic anhydride (0.7 mL) under N_2. After stirring for 45 min the reaction mixture was washed with satd aq $NaHCO_3$ solution, brine, dried over $MgSO_4$ and evaporated to an orange liquid. The obtained product 3-*O*-triflate-1:2,5:6-di-*O*-isopropylidene-α-D-galactofuranose **37** (318 mg, 0.86 mmol, 80%) was directly dissolved in dry DMF (2.5 mL) and cooled to 0 °C and sodium azide (244 mg, 3.75 mmol) was added. After stirring for 18 h at room temperature the reaction mixture was diluted with DCM, washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the obtained residue by column chromatography (EtOAc–hexane 1:1) afforded product **25** as a pale yellow solid (125 mg, 0.48 mmol, 55.4%). δ_H ($CDCl_3$, 300 MHz) 5.55 (1H, d, $J_{1,2}$ 4.9 Hz, H-1), 4.63 (1H, dd, *J* 2.3 Hz, *J* 7.8 Hz, H-3), 4.33 (1H, dd, *J* 2.3 Hz, *J* 4.9 Hz, H-2), 4.20 (1H, dd, *J* 1.8 Hz, *J* 7.7 Hz, H-4), 3.91 (1H, dd, J' 1.5 Hz, *J* 7.3 Hz, H-5), 3.51 (1H, dd, J' 8.0 Hz, *J* 12.7 Hz, H-6), 3.36 (1H, dd, J' 5.1 Hz, *J* 12.7 Hz, H-6′), 1.55 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.34 (6H, s, $(CH_3)_2CO_2$). δ_C ($CDCl_3$, 100 MHz): 96.5 (C-1), 70.8 (C-3), 70.5 (C-2), 71.4 (C-4), 67.0 (C-5), 50.9 (C-6), 26.8, 26.5, 26.2, 24.9 $(CH_3)_2CO_2$). ESI-HRMS: calcd for $C_{12}H_{20}N_3O_5$ $[M+H]^+$: 286.1325, found: 286.1340.

4.2.3. 1,2,4,6-Tetra-*O*-acetyl-3-azido-3-deoxy-α,β-D-gulopyranose 17

A solution of azido **25** (125 mg, 0.48 mmol) in 80% aqueous TFA (1 mL) was stirred at room temperature for 1 h. The mixture was then evaporated in vacuo and to the resulting oil were added pyridine (3 mL) and acetic anhydride (2 mL). After stirring for 20 h at room temperature, the solvents were removed under reduced pressure and the residue was diluted with DCM. The solution was washed with 1 M HCl, and satd aq $NaHCO_3$ solution, dried over $MgSO_4$, concentrated under reduced pressure and purified by column chromatography (EtOAc–hexane 1:1) to give product **17** (142 mg, 0.38 mmol, 80%) as a mixture of anomers α:β (0.5:1). δ_H ($CDCl_3$, 300 MHz). β anomer: 5.71 (1H, d, $J_{1,2}$ 8.2 Hz, H-1), 5.38–5.30 (1H, dd, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 10.4 Hz, H-2; 1H, m, H-4), 5.08 (1H, dd, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.4 Hz, H-3), 3.95 (1H, m, H-6), 3.45 (1H, dd, $J_{5,6'}$ 7.4 Hz, $J_{6,6'}$ 12.9 Hz, H-6′), 3.20 (1H, dd, $J_{4,5}$ 1.2 Hz, $J_{5,6}$ 5.4 Hz, H-5), 2.19–1.99 (12H, 4 s, $COCH_3$). δ_C ($CDCl_3$, 100 MHz) 170.8,

169.6, 168.5 ($COCH_3$), 85.7 (C-1), 72.5 (C-5), 67.2 (C-2), 65.2 (C-4), 60.2 (C-6), 58.9 (C-3), 21.3–20.7 ($COCH_3$).

α anomer: 6.40 (1H, d, $J_{1,2}$ 3.0 Hz, H-1), 5.48 (1H, m, H-4), 5.42–5.32 (2H, m, H-2, H-3), 4.23 (1H, m, H-6), 3.53 (1H, dd, $J_{5,6'}$ 7,4 Hz, $J_{6,6'}$ 12.9 Hz, H-6′), 3.24 (1H, dd, $J_{4,5}$ 1.2 Hz, $J_{5,6}$ 5.4 Hz, H-5), 2.19–1.99 (12H, 4s, $COCH_3$). IR (KBr) max 2935, *2112*, 1759, 1410, 1254 cm^{-1}. ESI-HRMS: calcd for $C_{14}H_{19}N_3O_9Na$ $[M+Na]^+$: 396.1121, found: 396.1114.

4.2.4. Azido-L-threonine benzyl ester 18

The amino acid *N*-(9-fluorenylmethoxycarbonyl)-L-threonine benzyl ester **26** (300 mg, 0.69 mmol, prepared from commercially available amino acid Fmoc-L-threonine by treatment with cesium carbonate and benzyl bromide in DMF)[59] was treated with 20% piperidine/DMF (1 mL) and stirred for 20 min at room temperature. After concentration in vacuo the residue was purified by column chromatography (EtOAc/hexane 7:3 v/v; MeOH/DCM 1:9 v/v). The obtained product L-threonine benzyl ester **27** (112.6 mg, 0.54 mmol, 78%) was submitted to diazo transfer reaction utilizing the method described by Wong et al.[41] Triflyl azide preparation: Sodium azide (0.6 g, 9.23 mmol) was dissolved in distilled water (2.0 mL) with DCM (2.5 mL) and cooled on an ice bath. Triflyl anhydride (0.3 mL, 1.79 mmol) was added slowly over 5 min while stirring continued for 2 h. The mixture was placed in a separatory funnel and the DCM phase was removed. The aqueous portion was extracted with DCM and the organic fractions, containing the triflyl azide, were pooled and washed once with saturated $NaHCO_3$ and used without further purification. Subsequently, L-threonine benzyl ester **27** (112.6 mg, 0.54 mmol) was dissolved in water and treated with $CuSO_4$ (8.6 mg, 0.05 mmol). Methanol (4 mL) and previously prepared triflyl azide in DCM (6 mL) were then added, and the solution was stirred at room temperature overnight. Solvents were then removed under reduced pressure to give a blue-green residue which was suspended in DCM (2 ml) and filtered through a plug of silica gel (3 cm in a Pasteur pipette); silica gel was washed with additional DCM (10 ml) and the combined filtrates were concentrated to give the product **18** as a pale oil in 60% yield (97.3 mg, 0.41 mmol). δ_H ($CDCl_3$, 500 MHz) 7.39–7.33 (5H, m, OCH_2Ph), 5.25 (2H, AB, J_{AB} = 12.2 Hz, OCH_2Ph), 4.26 (1H, dd, *J* 6.4 Hz, *J* 3.8 Hz, βC*H*Thr), 3.83 (1H, d, *J* 3.8 Hz, α*CH*Thr), 1.27 (3H, d, *J* 6.4 Hz, CH_3Thr). δ_C (100 MHz, $CDCl_3$): 169.5 ($COCH_2Ph$), 135.23 (*C*quat. OCH_2Ph), 129.1–128.8 (*C*HPh), 68.13 (OCH_2Ph), 68.9 (βCHThr), 67.6 (α*C*HThr), 20.3 (CH_3 Thr). IR (KBr) max 3450, *2109*, 1600, 1100 cm^{-1}. ESI-HRMS: calcd for $C_{14}H_{13}N_3O_3Na$ $[M+Na]^+$: 258.0957, found: 258.0948.

4.2.5. 2-Propynyl 5-acetamido-3,5-dideoxi-4,7,8,9-tetra-*O*-acetyl-D-*glycerol*-α-D-*galacto*-non-2-ulopyranoside methyl ester 19

Method A. β-Acetochloroneuraminic acid **28** (221 mg, 0.43 mmol), propargyl alcohol (0.86 mmol, 50 μL) and 4 Å molecular sieves (200 mg) in dry DCM (5 mL) was stirred at −20 °C under N_2 during 1 h before adding AgOTf (132.6 mg, 0.52 mmol) in dry toluene (1 mL). The reaction was allowed to warm up to room temperature. After 2 h, the solution was neutralized with triethylamine, filtered through Celite and concentrated in vacuo. Column chromatography (EtOAc–Hexane 7:3 v:v) afforded the product **19** as a pale yellow amorphous solid in 66% yield (150.3 mg, 0.28 mmol).

Method B. To a solution of the per-acetate sialic acid **29** (117 mg, 0.22 mmol) in dry DCM (2.0 mL) was added propargyl alcohol (0.26 mmol, 16 μL) and BF_3. Et_2O (0.33 mmol, 40 μL) at 0 °C, being the reaction mixture stirred at room temperature for 5 h. The resultant solution was washed successively with dilute HCl solution, satd $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated. Column chromatography (EtOAc–Hexane 1:1 v:v) afforded the product **19** as a pale yellow amorphous solid in 70% yield (82 mg, 0.15 mmol, 70%). $[\alpha]_D$ +69.4 (*c* 0.5, CH_3CN). δ_H ($CDCl_3$, 300 MHz) 5.60 (1H, d, $J_{5,NH}$, 9.6 Hz, NH), 5.47 (1H, dd, $J_{8,9'}$ 3.2 Hz, $J_{8,9}$ = $J_{7,8}$ 6.8 Hz, H-8), 5.31 (1H, dd, $J_{6,7}$ 2.9 Hz, $J_{7,8}$ 7.3 Hz, H-7), 4.62 (1H, dd, $J_{3a,4}$ 3.1 Hz, $J_{3e,4}$ 12.3 Hz, H-4), 4.41–4.34 (1H, dd, *J* 2.4 Hz, *J* 15.7 Hz, $CH_{2a}C{\equiv}CH$), 4.27 (1H, dd, $J_{8,9'}$ 2.7 Hz, $J_{9,9'}$ 12,4 Hz, H-9′), 4.21–4.02 (4H, m, H-5, H-6, H-9, $CH_{2b}C{\equiv}CH$), 3.77 (3H, s, CO_2CH_3), 2.60 (1H, dd, $J_{3e,4}$ 4,6 Hz, $J_{3a,3e}$ 13,7 Hz, H-3e), 2.43 (1H, t, *J* 2.4 Hz, $CH_2C{\equiv}CH$), 2.12–1.98 (4 × 3H, 4s, 4 × $OCOCH_3$), 1.95 (3H, s, $NHCOCH_3$), 1.91 (1H, dd, $J_{3a,4}$ 11.2, $J_{3a,3e}$ 13.7, H-3a). δ_C (100 MHz, $CDCl_3$): 171.0, 170.7, 170.6, 170.3, 170.0 (4 × $OCOCH_3$ and CONH), 166.8 (C-1), 98.7 (C-2), 79.0 (*C*≡CH), 75.3 (C≡*C*H), 72.4 (C-6), 68.7 (C-4), 68.3 (C-8), 67.3 (C-7), 62.4 (C-9), 52.8 (OCH_3), 52.2 ($OCH_2C{\equiv}$), 49.2 (C-5), 37.1 (C-3), 23.1 ($NHCOCH_3$), 21.0, 20.8, 20.7, 20.6 (4 × $OCOCH_3$).

ESI-HRMS: calcd for $C_{23}H_{31}NO_{13}Na$ $[M+Na]^+$: 552.1795, found: 552.1706.

4.2.6. Methyl 3-prop-2-ynyl-α-D-galactopyranoside 31

Methyl-α-D-galactopyranose **30** (500 mg, 2.57 mmol) was dissolved in MeOH (20 mL). Subsequently, bis-dibutyltin oxide (671.7 mg, 2.69 mmol) was added and the reaction mixture was heated under reflux for 2 h. Then, the solvent was evaporated and the residual solid was dried in vacuo for several hours. The obtained white solite was diluted with toluene (20 mL) and treated with tetrabutylammonium iodide (949.3 mg, 2.57 mmol) and propargyl bromide (1.2 g, 10.28 mmol, 0.9 mL). The reaction was stirred at room temperature for 12 h, then filtered through a silica path. The resulting organic solution was concentrated under reduced pressure and purified by column chromatography (EtOAc–Hexane 7:3 v:v) to afford **31** (482.5 mg, 2.07 mmol, 81%) as an yellow oil. δ_H ($CDCl_3$, 500 MHz), 4.72 (1H, d, $J_{1,2}$ 3.8 Hz, H-1), 4.29 (2H, dd, *J* 2.5 Hz, *J* 15.8 Hz, $CH_2C{\equiv}CH$), 4.10 (1H, d, *J* 2.8, H-4), 3.86 (1H, dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.8 Hz, H-2), 3.76 (1H, dd, $J_{5,6}$ 7.7 Hz, $J_{6,6'}$ 12.0 Hz, H-6), 3.72–3.68 (2H, m, H-6′, H-5), 3,65 (1H, dd, $J_{3,4}$ 31 Hz, $J_{2,3}$ 9.8 Hz, H-3), 3.35 (3H, s, OCH_3), 2,44 (1H, t, *J* 2.3 Hz, $CH_2C{\equiv}CH$). δ_C (100 MHz, $CDCl_3$): 99.9 (C-1), 78.9 (*C*≡CH), 78.0 (C-3), 74.4 (C≡*C*H), 69.2 (C-5), 68.5 (C-4), 63.4 (C-6), 63.1 (C-2), 57.4 ($OCH_2C{\equiv}$), 55.18 (OCH_3).

ESI-HRMS: calcd for $C_{10}H_{16}O_6Na$ $[M+Na]^+$: 255.0947, found: 255.0958.

4.2.7. Methyl 2,4,6-tri-*O*-acetyl-3-prop-2-ynyl-α-D-galactopyranoside 20

A solution of the sugar **31** (88 mg, 0.37 mmol), anhydrous pyridine (2.7 mL) and acetic anhydride (2 mL) was stirred at room temperature for 20 h. After removal of solvents, the residue was dissolved in DCM and the solution was washed with 1 M HCl, and satd aq $NaHCO_3$ solution, dried over Mg_2SO_4, concentrated under reduced pressure and purified by column chromatography (EtOAc–Hexane 1:1) to give **20** as a pale oil (135.74 mg, 0.37 mmol, 100%). δ_H ($CDCl_3$, 500 MHz) 5.47 (1H, d, *J* 3.3, H-4), 5.07 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.3 Hz, H-2), 4.96 (1H, d, $J_{1,2}$ 3.8 Hz, H-1), 4.20 (2H, dd, *J* 2.3 Hz, *J* 16.0 Hz, $CH_2C{\equiv}CH$), 4.14 (1H, dd, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 12.19 Hz, H-6), 4.13–4.08 (3H, m, H-6′, H-5, H-3), 3.40 (3H, s, OCH_3), 2.45 (1H, t, *J* 2.3 Hz, $CH_2C{\equiv}CH$), 2.14–2.07 (9H, 3s, $COCH_3$). δ_C (100 MHz, $CDCl_3$): 170.9, 170.8, 170.7 ($COCH_3$), 98.0 (C-1), 79.5 (*C*≡CH), 75.3 (C≡*C*H), 72.6 (C-3), 69.8 (C-2), 67.5 (C-4), 66.8 (C-5), 62.8 (C-6), 57.3 ($CH_2C{\equiv}CH$), 55.8 (OCH_3), 21.4, 21.1, 20.9 ($COCH_3$).

ESI-HRMS: calcd for $C_{16}H_{22}O_9Na$ $[M+Na]^+$: 381.1264, found: 381.1156.

4.3. General procedure for 'click chemistry' reactions

A solution of 2-propynyl-sialic acid **18**/3-*O*-propynyl-GalOMe **20** (1 equiv), azido-functionalized sugars **14**–**17**/amino acid **18**

(1 equiv), sodium ascorbate (0.5 equiv) and $CuSO_4$ (0.1 equiv) in DMF (0.5 mL) was placed into a microwave tube. Then, the tube was sealed and submitted to microwave irradiation in 15 min bursts (100 °C, 50 W). The reaction was followed by TLC (EtOAc) taking samples at 15 min intervals. After completion the reaction mixture was partitioned between H_2O and EtOAc and the aqueous phase was extracted with EtOAc. The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure and purified by column chromatography (EtOAc/EtOAc–Hexane 7:3 v:v) to afford the desired 1,2,3-triazole sialylmimetic neoglycoconjugates **32–36**.

4.3.1. Methyl {5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-2-oxymethyl-[1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1*H*-1,2,3-triazol-4-yl]-D-*glycerol*-α-D-*galacto*-non-2-ulopyranoside}onate 32

Following procedure described in Section 4.3, the reaction of 2-propynyl-sialic acid **19** (50 mg, 0.09 mmol), azido-2,3,4,6-tetra-*O*-acetyl-1-deoxy-α-D-galactopyranose **14** (35 mg, 0.09 mmol), sodium ascorbate (9.31 mg, 0.05 mmol) and $CuSO_4$ (1.5 mg, 9.4 μmol, 9.4 μL of 1 M solution) in DMF (0.5 mL) afforded the product **32** as a pale yellow solid (27.5 mg, 0.03 mmol, 32.4%). δ_H (CD_3OD, 300 MHz) 8.16 (1H, s, CH triazole), 6.11 (1H, d, $J_{1,2}$ 9.3 Hz, H-1), 5.69 (1H, dd, $J_{1,2}$ 9.2 Hz, $J_{2,3}$ 10.14 Hz, H-2), 5.56 (1H, d, $J_{3,4}$ 2.8 Hz, H-4), 5.48 (1H, m, H-8), 5.42 (1H, dd, $J_{3'a,4}$ 3.2 Hz, $J_{2,3}$ 10.14 Hz, H-3), 5.35 (1H, dd, $J_{6,7}$ 1.8 Hz, $J_{7,8}$ 9.2 Hz, H-7), 4.80–4.63 (2H, m, CH_aH_b-triazole), 4.58 (1H, dd, $J_{3'a,4}$ 4.9 Hz, $J_{3'e,4}$ 12.3 Hz, H-4′), 4.48 (1H, t, J 5.9 Hz, H-5′), 4.32 (1H, dd, $J_{8,9}$ 2.6 Hz, $J_{9,9'}$ 12.4 Hz, H-9), 4.21 (1H, m, H-6′), 4.16–4.05 (3H, m, H-6a, H-6b, H-9′), 4.0 (1H, dd, $J_{4,5}$ 7.3 Hz, $J_{5,6}$ 9.6, H-5), 3.77 (3H, s, CO_2CH_3), 2.66 (1H, dd, $J_{3'e,4}$ 4.6 Hz, $J_{3'a,3'e}$ 12.7 Hz, H-3′e), 2.23–1.95 (8 × 3H, 8s, 8 × OCOC*H*$_3$), 1.89 (3H, s, NHCOC*H*$_3$), 1.87 (1H, dd, $J_{3'a,4}$ 5.1 Hz, $J_{3'a,3'e}$ 13.5 Hz, H-3′a). δ_C (100 MHz, $CDCl_3$): 172.1–168.1 (8 × O*C*OCH$_3$ and *C*ONH), 123.1 (*C*H triazole), 85.7 (C-1), 73.9 (C-5′), 70.6 (C-8), 67.9 (C-2), 67.8 (C-3), 67.4 (C-4), 67.3 (C-7), 62.1 (C-9), 61.5 (C-6′), 60.6 (C-6), 57.4 (CH_aH_b-triazole), 57.1 (C-4′), 52.06 (O*C*H$_3$), 48.5 (C-5), 37.1 (C-3′), 21.3 (NHCO*C*H$_3$), 19.9–18.8 (8 × OCO*C*H$_3$). ESI-HRMS: calcd for $C_{37}H_{50}N_4O_{22}$ Na $[M+Na]^+$: 925.2917, found: 925.2867.

4.3.2. Methyl {5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-2-oxymethyl-[1-(1,2,3,4-tetra-*O*-acetyl-α-D-galactopyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]-D-*glycerol*-α-D-*galacto*-non-2-ulopyranoside}onate 33

Following procedure described in Section 4.3, the reaction of 2-propynyl-sialic acid **19** (50 mg, 0.09 mmol), 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-α-D-galactopyranose **15** (35 mg, 0.09 mmol), sodium ascorbate (9.31 mg, 0.05 mmol) and $CuSO_4$ (1.5 mg, 9.4 μmol, 9.4 μL of 1 M solution) in DMF (0.5 mL) afforded the product **33** as a pale yellow solid (29 mg, 0.03 mmol, 34%). δ_H (CD_3OD, 300 MHz) 7.97 (1H, s, CH triazole), 5.68–4.92 (8H, m, H-1, H-4, H-3, H-2, H-8, H-7, CH_aH_b-triazole), 4.58 (1H, dd, $J_{3'a,4}$ 6.2 Hz, $J_{3'e,4}$ 11.7 Hz, H-4′), 4.48 (1H, dd, $J_{5',6a}$ 2.0 Hz, $J_{6a,6b}$12.7 Hz, H-6a), 4.32 (1H, dd, $J_{8,9}$ 2.4 Hz, $J_{9,9'}$ 12.3 Hz, H-9), 4.25 (1H, m, H-5), 4.20 (1H, dd, $J_{5',6b}$ 2.2 Hz, $J_{6a,6b}$ 10.8 Hz, H-6b), 4.09–3.94 (3H, m, H-5′, H-9′, H-6′), 3.82 (3H, s, CO_2CH_3), 2.65 (1H, dd, $J_{3'e,4}$ 4.8 Hz, $J_{3'a,3'e}$ 12.3 Hz, H-3′e), 2.17–1.91 (8 × 3H, 8s, 8 × OCOC*H*$_3$), 1.89 (3H, s, NHCOC*H*$_3$), 1.86 (1H, dd, $J_{3'a,4}$ 5.1 Hz, $J_{3'a,3'e}$ 13.5 Hz, H-3′a). δ_C (100 MHz, $CDCl_3$): 172.5–167.9 (8 × O*C*OCH$_3$ and *C*ONH), 125.3 (*C*H triazole), 92.05 (C-1), 70.3 (C-5), 70.6 (C-8), 68.2 (C-4), 68.1 (C-2), 67.6 (C-3), 67.0 (C-7), 62.1 (C-9), 61.7 (C-6′), 57.4 (C-6), 57.7 (CH_aH_b-triazole), 51.6 (O*C*H$_3$), 49.5 (C-4′), 47.9 (C-5′), 37.4 (C-3′), 21.6 (NHCO*C*H$_3$), 21.4–19.2 (8 × OCO*C*H$_3$). ESI-HRMS: calcd for $C_{37}H_{52}N_4O_{22}$ $[M+H]^+$: 903.2917, found: 903.2986.

4.3.3. Methyl {5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-2-oxymethyl-[1-(1,2,4,6-tetra-*O*-acetyl-α-D-glucopyranosid-3-yl)-1*H*-1,2,3-triazol-4-yl]-D-*glycerol*-α-D-*galacto*-non-2-ulopyranoside}onate 34

Following procedure described in Section 4.3, the reaction of 2-propynyl-sialic acid **19** (30 mg, 0.05 mmol), 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-α,β-D-glucopyranose **16** (35 mg, 0.09 mmol), sodium ascorbate (5.54 mg, 0.03 mmol) and $CuSO_4$ (0.9 mg, 5.6 μmol, 6 μL of 1 M solution) in DMF (0.5 mL) afforded the product **34** as a pale yellow solid (38 mg, 0.04 mmol, 74%). (CD_3OD, 300 MHz) 8.3 (1H, s, CH triazole), 6.42 (1H, d, $J_{1,2}$ 3.5 Hz, H-1), 5.72 (1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 11.2 Hz, H-2), 5.43 (1H, dd, $J_{8,9}$ 2.0 Hz, $J_{8,9'}$ 4.8 Hz, H-8), 5.37 (1H, dd, $J_{6,7}$ 2.3 Hz, $J_{7,8}$ 9.3 Hz, H-7), 5.25 (1H, t, J 10.4 Hz, H-3), 5.15 (1H, dd, $J_{3'a,4'}$ 3.9 Hz, $J_{3'e,4}$ 12.9 Hz, H-4′), 4.82–4.66 (2H, m, CH_aH_b-triazole), 4.42–4.30 (2H, m, H-6a, H-9), 4.20 (1H, dd, $J_{5,6b}$ 2.1 Hz, $J_{6a,6b}$ 10.6 Hz, H-6b), 4.14–4.03 (4H, m, H-5, H-5′, H-6′, H-9′), 3.99 (1H, $J_{3,4}$ 3.1, H-4), 3.82 (3H, s, CO_2CH_3), 2.42 (1H, dd, $J_{3'e,4}$ 4.2 Hz, $J_{3'a,3'e}$ 12.17 Hz, H-3′e), 2.17–1.91 (8 × 3H, 8s, 4 × OCOC*H*$_3$), 1.89 (3H, s, NHCOC*H*$_3$), 1.87 (1H, dd, $J_{3'a,4'}$ 4.9 Hz, $J_{3'a,3'e}$ 14.3 Hz, H-3′a). δ_C (100 MHz, $CDCl_3$): 172.1–167.3 (8 × O*C*OCH$_3$ and *C*ONH), 124.2 (*C*H triazole), 88.1 (C-1), 70.5 (C-7), 68.1 (C-4′), 67.9 (C-2), 67.5 (C-8), 61.3 (C-9), 61.6 (C-5), 61.2 (C-5′), 60.9 (C-3), 60.7 (C-6′), 60.3 (C-6), 56.8 (CH_aH_b-triazole), 51.4 (C-4), 51.9 (O*C*H$_3$), 36.4 (C-3′), 21.4 (NHCO*C*H$_3$), 19.7–18.7 (8 × OCO*C*H$_3$). ESI-HRMS: calcd for $C_{37}H_{50}N_4O_{22}Na$ $[M+Na]^+$: 925.2917, found: 925.2878.

4.3.4. Methyl {5-acetamido-3,5-dideoxy-4,7,8,9-tetra-*O*-acetyl-2-oxymethyl-[1-(1,2,4,6-tetra-*O*-acetyl-β-D-gulopyranosid-3-yl)-1*H*-1,2,3-triazol-4-yl]-D-*glycerol*-α-D-*galacto*-non-2-ulopyranoside}onate 35

Following procedure described in Section 4.3, the reaction of 2-propynyl-sialic acid **19** (26.5 mg, 0.05 mmol), 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-α,β-D-gulopyranose **17** (18.7 mg, 0.05 mmol), sodium ascorbate (5.0 mg, 0.02 mmol) and $CuSO_4$ (0.8 mg, 5.0 μmol, 5 μL of 1 M solution) in DMF (0.5 mL) afforded the product **35** as a pale yellow solid (19.6 mg, 0.02 mmol, 43.4%). (CD_3OD, 300 MHz) 7.98 (1H, s, CH triazole), 5.78 (1H, d, $J_{1,2}$ 8.1 Hz, H-1), 5.52 (1H, d, $J_{3,4}$ 3.1, H-4), 5.44 (1H, m, H-7), 5.39–5.30 (2H, m, H-3, H-8), 5.25 (1H, dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.3 Hz, H-2), 5.17 (1H, m, H-4′), 4.80–4.73 (2H, m, CH_aH_b-triazole), 4.70–4.52 (2H, m, H-6a, H-9), 4.26–4.18 (2H, m, H-6b, H-6′), 4.11–3.96 (3H, m, H-5, H-9′, H-5′), 3.82 (3H, s, CO_2CH_3), 2.46 (1H, dd, $J_{3'e,4'}$ 5.14 Hz, $J_{3'a,3'e}$ 13.10 Hz, H-3′e), 2.19–1.94 (8 × 3H, 8s, 4 × OCOC*H*$_3$), 1.85 (3H, s, NHCOC*H*$_3$), 1.84 (1H, dd, $J_{3'a,4'}$ 4.8 Hz, $J_{3'a,3'e}$ 13,1 Hz, H-3′a). δ_C (100 MHz, $CDCl_3$): 172.1–167.3 (8 × O*C*OCH$_3$ and *C*ONH), 124.3 (*C*H triazole), 70.5 (C-4′), 68.7 (C-3), 68.4 (C-8), 67.9 (C-7), 67.7 (C-1), 68.5 (C-4), 66.9 (C-2), 61.9 (C-9), 61.7 (C-6), 61.6 (C-6′), 60.8 (C-5′), 56.5 (CH_aH_b-triazole), 50.3 (O*C*H$_3$), 47.9 (C-5), 37.2 (C-3′), 21.7 (NHCO*C*H$_3$), 19.6–18.8 (8 × OCO*C*H$_3$). ESI-HRMS: calcd for $C_{37}H_{50}N_4O_{22}Na$ $[M+Na]^+$: 925.2917, found: 925.2871.

4.3.5. Methyl 2,4,6-tri-*O*-acetyl-3-oxymethyl-[1-(3-hydroxy)-butanoic acid)-1*H*-1,2,3-triazol-4-yl]-α-D-galactopyranoside benzyl ester 36

Following procedure described in Section 4.3, the reaction of azido-L-threonine benzyl ester **18** (37.2 mg, 0.10 mmol), methyl-2,4,6-tri-*O*-acetyl-3-prop-2-ynyl-α-D-galactopyranose **20** (24.4 mg, 0.10 mmol), sodium ascorbate (10.20 mg, 0.05 mmol) and $CuSO_4$ (0.01 mmol, 0.1 equiv) in DMF (0.5 mL) gave the product **36** as a pale yellow solid (29.3 mg, 0.05 mmol, 48.3%) after purification by column chromatography (EtOAc–Hexane 7:3 v:v). δ_H ($CDCl_3$, 500 MHz) 7.97 (1H, s, C*H* triazole), 7.38–7.27 (5H, m, OCH$_2$Ph), 5.49 (1H, d, $J_{3,4}$ 3.6 Hz, H-4), 5.37 (1H, d, J 3.3 Hz, α*CH*Thr), 5.31–5.22 (2H, AB, J_{AB} = 12.2 Hz, OC*H*$_2$Ph), 5.04 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.3 Hz, H-2), 4.96 (1H, d, $J_{1,2}$

3.6 Hz, H-1), 4.84–4.69 (2H, d, *J* 12.4 Hz, CH_aH_b-triazole), 4.68 (1H, m, βC*H*Thr), 4.12–4.02 (3H, m, H-6, H-6′, H-5), 3.98 (1H, dd, $J_{3,4}$ 3.3 Hz, $J_{2,3}$ 10.3 Hz, H-3), 3.37 (3H, s, OCH_3), 2.11–2.06 (9H, 3s, COCH_3), 1.06 (3H, d, *J* 6.4 Hz, CH_3Thr). δ_C (100 MHz, $CDCl_3$) 171.5, 170.9, 170.8 (*C*OCH$_3$), 169.5 (*C*OCH$_2$Ph), 135.23 (*C*quat. OCH$_2$Ph), 129.1–128.2 (*C*HPh), 124.6 (*C*H triazole), 97.1 (C-1), 73.5 (C-3), 69.6 (C-2), 67.6 (C-4), 67.4 (OCH$_2$Ph), 66.8 (α*C*HThr), 66.5 (C-5), 64.3 (CH_aH_b-triazole), 63.7 (β*C*HThr), 61.8 (C-6), 55.4 (OCH$_3$), 21.4–21.0 (COCH$_3$).

ESI-HRMS: calcd for $C_{27}H_{36}N_3O_{12}$ $[M+H]^+$: 594.2221, found: 594.2286.

4.3.6. General procedure for deprotection reactions of compounds 32–35

To a solution of **32–35** in methanol (0.5 mL) was added 1 M NaOMe until pH 9–10 was achieved. The mixture was stirred for 3 h at room temperature, neutralized with ion exchange resin (Dowex 50WX8-200H$^+$), filtered and concentrated under reduced pressure. The obtained product was treated with 0.2 M KOH (0.5 mL) and the solution was stirred for 12 h at room temperature. After neutralization with ion exchange resin (Dowex 50WX8–200H$^+$) the solution was filtered and the solvent was removed under reduced pressure. The obtained deprotected compounds **9**–**12** were purified by HPLC on a semi-preparative C18 column (Shim-PaK CLC-ODS (M)) using gradient elution with 0.1% aq CF_3CO_2H (A)–CH_3CN (B), 0–100% B in 25 min, with detection of peaks at 252 nm with a UV detector.

4.3.7. 5-Acetamido-3,5-dideoxy-2-oxymethyl-[1-(β-D-galacto-pyranosyl)-1*H*-1,2,3-triazol-4-yl]-D-*glycerol*-α-D-*galacto*-non-2-ulopyranosidic acid 9

Compound **32** (23 mg, 0.025 mmol) was reacted as described in the general procedure (Section 4.3.6). The deprotected product **9** was obtained as colourless amorphous solid in 13% yield (1.8 mg, 3.2 μmol) after HPLC purification. δ_H (CD_3OD, 300 MHz) 8.14 (1H, s, CH triazole), 5.56 (1H, d, $J_{1,2}$ 9.3 Hz, H-1), 4.80 (1H, d, *J* 12.0 Hz, CH_{2a} triazole), 4.53 (1H, d, *J* 12.0 Hz, CH_{2b} triazole), 4.08 (1H, dd, $J_{1,2}$ 9.2 Hz, $J_{2,3}$ 10.14 Hz, H-2), 3.95 (1H, d, *J* 2.8 Hz, H-4), 3.90–3.80 (2H, m, H-8, H-6′), 3.78–3.70 (3H, m, H-3, H-5, H-9), 3.68–3.61 (3H, m, H-7, H-5′, H-9′), 3.54–3.43 (3H, m, H-4′, H-6a, H-6b), 2.61 (1H, dd, $J_{3'e,4'}$4.6 Hz, $J_{3'a,3'e}$ 12.6 Hz, H-3′e), 1.90 (3H, s, NHCOCH_3), 1.55 (1H, t, $J_{3'a,3'e}$ 12.0 Hz, H-3′a). δ_C (100 MHz, $CDCl_3$) 173.2 (*C*ONH), 124.0 (*C*H triazole), 88.0 (C-1), 78.0 (C-8), 72.8 (C-9), 71.9 (C-7), 69.7 (C-6′), 69.5 (C-2), 68.2 (C-6), 68.1 (C-4), 62.7 (C-3), 62.15 (C-4′), 57.5 (CH_aH_b-triazole), 40.0 (C-3′), 21.8 (NHCO*C*H$_3$).

ESI-HRMS: calcd for $C_{20}H_{33}N_4O_{14}$ $[M+H]^+$: 553.1915, found: 553.1987.

4.3.8. 5-Acetamido-3,5-dideoxy-2-oxymethyl-[1-(α-D-galacto-pyranosid-6-yl)-1*H*-1,2,3-triazol-4-yl]-D-*glycerol*-α-D-*galacto*-non-2-ulopyranosidic acid 10

Compound **33** (12 mg, 0.013 mmol) was reacted as described in the general procedure (Section 4.3.6). The deprotected product **10** was obtained as colourless amorphous solid in 45% yield (3.3 mg, 5.9 μmol) after HPLC purification. δ_H (CD_3OD, 300 MHz) 7.95 (1H, s, C*H* triazole), 4.81 (1H, d, *J* 12.0 Hz, CH_a triazole), 4.59 (1H, d, *J* 12.0 Hz, CH_b triazole), 4.51 (1H, d, $J_{1,2}$ 3.9 Hz, H-1) 4.50–3.80 (6H, m, H-4, H-8, H-7, H-5′, H-3, H-2), 3.77–3.70 (2H, m, H-9, H-9′), 3.65 (1H, dd, $J_{3'a,4'}$ 4.9 Hz, $J_{3'e,4}$ 12.3 Hz, H-4′), 3.57–3.43 (4H, m, H-5, H-6′, H-6a, H-6b), 2.60 (1H, dd, $J_{3'e,4'}$ 4.9 Hz, $J_{3'a,3'e}$ 12.9 Hz, H-3′e), 1.91 (3H, s, NHCOCH_3), 1.66 (1H, t, *J* 13.41 Hz, H-3′a). δ_C (100 MHz, $CDCl_3$) 173.0 (*C*ONH), 123.7 (*C*H triazole), 74.2 (C-1), 70.25 (C-8), 69.9 (C-2), 69.2 (C-7), 68.2 (C-6′), 67.7 (C-4′), 67.4 (C-5), 66.3 (C-3), 63.2 (C-9), 62.7 (C-6), 58.9 (C-5′), 53.8 (CH_aH_b-triazole), 52.0 (C-4), 38.7 (C-3′), 21.5 (NHCO*C*H$_3$).

ESI-HRMS: calcd for $C_{20}H_{33}N_4O_{14}$ $[M+H]^+$: 553.1915, found: 553.1983.

4.3.9. 5-Acetamido-3,5-dideoxy-2-oxymethyl-[1-(α-D-glucopyranosid-3-yl)-1*H*-1,2,3-triazol-4-yl]-D-*glycerol*-α-D-*galacto*-non-2-ulopyranosidic acid 11

Compound **34** (37 mg, 0.04 mmol) was reacted as described in the general procedure (Section 4.3.6). The deprotected product **11** was obtained as colourless amorphous solid in 15.4% yield (3.4 mg, 6.1 μmol) after HPLC purification. δ_H (CD_3OD, 300 MHz) 8.19 (1H, s, C*H* triazole), 5.38 (1H, d, *J* 3.6 Hz, H-1), 4.73 (1H, d, *J* 11.67 Hz, CH_a triazole), 4.50 (1H, d, *J* 11.67 Hz, CH_b triazole), 4.23 (1H, dd, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.6 Hz, H-2), 4.04 (1H, m, H-4′), 4.02–3.74 (6H, m, H-8, H-7, H-5, H-9a, H-6′, H-5′), 3.68 (2H, dd, $J_{5,6'}$5.7 Hz, $J_{6,6'}$ 11.9 Hz, H-6a, H-6b), 3.58 (1H, m, H-9b), 2.38 (1H, dd, $J_{3'e,4'}$ 4.6 Hz, $J_{3'a,3'e}$ 13.2 Hz, H-3′e), 2.05 (3H, s, NHCOCH_3), 1.71 (1H, t, $J_{3'a,3'e}$ 11.6 Hz, H-3′a). δ_C (100 MHz, $CDCl_3$) 174.0 (*C*ONH), 124 (*C*H triazole), 85.8 (C-1), 74.4 (C-6′), 72.1 (C-2), 68.9 (C-9), 68.3 (C-4), 68.15 (C-5), 66.8 (C-4′), 63.3 (C-6), 62.8 (C-3), 56.2 (CH_aH_b-triazole), 52.3 (C-5′), 39.6 (C-3′), 21.8 (NHCO*C*H$_3$).

ESI-HRMS: calcd for $C_{20}H_{32}N_4O_{14}{\cdot}3NH_4Na$ $[M+3NH_4+Na]^+$ 629.1115, found 629.1106.

4.3.10. 5-Acetamido-3,5-dideoxy-2-oxymethyl-[1-(β-D-gulo-pyranosid-3-yl)-1*H*-1,2,3-triazol-4-yl]-D-*glycerol*-α-D-*galacto*-non-2-ulopyranosidic acid 12

Compound **35** (19 mg, 0.02 mmol) was reacted as described in the general procedure (Section 4.3.6). The deprotected product **12** was obtained as colourless amorphous solid in 47% yield (5.6 mg, 0.01 mmol) after HPLC purification. δ_H (CD_3OD, 300 MHz) 7.99 (1H, s, C*H* triazole), 4.59 (1H, d, *J* 11.4 Hz, CH_a triazole), 4.53 (1H, d, $J_{1,2}$ 8.3 Hz, H-1), 4.45 (1H, m, H-3), 4.39 (1H, d, *J* 10.9 Hz, CH_b triazole), 4.22 (1H, m, H-2), 4.07–3.81 (4H, m, H-7, H-8, H-4′, H-6′, H-9a), 3.78–3.73 (3H, m, H-6a, H-9b, H-4), 3.65–3.53 (1H, m, H-5, 1H, dd, $J_{5,6b}$ 5.7 Hz, $J_{6a,6b}$11.9 Hz, H-6b), 3.46 (1H, d, $J_{5',6'}$ 9.6 Hz, H-5′), 2.28 (1H, dd, $J_{3'e,4'}$ 4.1 Hz, $J_{3'a,3'e}$ 13.4 Hz, H-3′e), 1.94 (3H, s, NHCOCH_3), 1.59 (1H, t, $J_{3'a,3'e}$ 11.9 Hz, H-3′a). δ_C (100 MHz, $CDCl_3$) 174.0 (*C*ONH), 126.1 (*C*H triazole), 75.3 (C-1), 73.2 (C-7), 70.3 (C-8), 69.6 (C-2), 68.3 (C-9), 67.8 (C-5), 66.5 (C-4′), 63.9 (C-6′), 63.4 (C-4), 63.2 (C-6), 56.0 (CH_aH_b-triazole), 53.6 (C-3), 39.8 (C-3″), 21.7 (NHCO*C*H$_3$).

ESI-HRMS: calcd for $C_{20}H_{34}N_4O_{14}{\cdot}KNH_4$ $[M+K+NH_4+2H]^+$ 611.3515, found 611.3528.

4.3.11. Methyl 3-oxymethyl-[1-(3-hydroxy)-butanoic acid)-1*H*-1,2,3-triazole-4-yl]-α-D-galactopyranoside 13

A solution of compound **36** (29 mg, 0.05 mmol) in MeOH (0.5 mL) was treated with glacial AcOH (0.05 mL) and 10% Pd/C (10 mg) for removal of the *O*-Bn group. The reaction mixture was stirred and kept under H_2 (~1.5 atm) for 5 h. The reaction mixture was then filtered through Celite, concentrated in vacuo and purified by column chromatography (DCM–MeOH 9:1 v/v). The obtained product (19.4 mg, 0.04 mmol, 79%) was then dissolved in MeOH (0.5 mL) and made basic with 1 M NaOMe in MeOH. The reaction mixture was stirred for 3 h, and then neutralized with Dowex 50WX8-200 resin. Filtration and concentration of the reaction mixture gave the product **12** (11.32 mg, 0.03 mmol, 80%) as a white amorphous solid. δ_H ($CDCl_3$, 500 MHz) 8.17 (1H, s, C*H* triazole), 5.15 (1H, d, *J* 3.6 Hz, α*C*HThr), 4.77 (2H, dd, *J* 7.7 Hz, *J* 12.2 Hz, CH_aH_b-triazole), 4.73 (1H, d, $J_{1,2}$ 3.6 Hz, H-1), 4.64 (1H, m, β*C*HThr), 4.10 (1H, d, $J_{3,4}$ 3.0 Hz, H-4), 3.92 (1H, dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 10.1 Hz, H-2), 3.78–3.70 (3H, m, H-6, H-6′, H-5), 3.67 (1H, dd, $J_{3,4}$ 2.8 Hz, $J_{2,3}$ 9.8 Hz, H-3), 3.41 (3H, s, OCH_3), 1.06 (3H, d, *J* 6.4 Hz, CH_3Thr). δ_C (100 MHz, $CDCl_3$) 129.3 (*C*H triazole), 100.4 (C-1), 79.3 (C-3), 71.4 (α*C*HThr), 71.2 (C-5), 68.6 (C-2), 67.7 (β*C*HThr), 66.6 (C-4), 63.1 (CH_aH_b-triazole), 61.5 (C-6), 54.8 (OCH$_3$).

ESI-HRMS: calcd for $C_{13}H_{21}N_3O_9{\cdot}KNH_4$ $[M+K+NH_4]^+$: 434.2434, found: 434.2325.

- ^1H NMR and ESI-HRMS spectra of compounds **32–35** and **9–12** are presented in the Supplementary data (ESI)†.
- A figure related to the 2D Heteronuclear Correlation Experiment (*G-BIRD*$_{R,X}$*-CPMG-HSQMBC*), confirming the α-anomeric configuration of the obtained sialosides is showed in the ESI†.

4.4. Biological assays

4.4.1. Fluorimetric TcTS inhibition assays

trans-Sialidase used in this study was a His-tagged 70 kDa recombinant material truncated to remove C-terminal repeats but retaining the catalytic N-terminal domain of the enzyme.[58] Inhibition was assessed using the continuous fluorimetric assay described by Douglas and co-workers.[53] Briefly, the assay was performed in triplicate in 96-well plates containing phosphate buffer solution at pH 7.4 (25 μL), recombinant enzyme solution (25 μL) and inhibitor solution (25 μL of 4.0 mM/2.0 mM solution). This mixture was incubated for 10 min at 26 °C followed by addition of MuNANA (K_m = 0.68 mM[53]; 25 μL of a 0.4 mM solution giving an assay concentration of 0.1 mM). The fluorescence of the released product (Mu) was measured after 10 min, with excitation and emission wavelengths of 360 and 460 nm, respectively, and the data were analyzed with GraphPad Prism software version 4.0 (San Diego, CA, USA). Inhibition percentages were calculated by the equation: $\%\ I = 100 \times [1 - (V_i/V_0)]$, where V_i is the velocity in the presence of inhibitor and V_0 is the velocity in absence of inhibitor.

- A Figure presenting the inhibition curves for TcTS inhibition assays by compounds **9–13** and DANA is showed in the ESI†.

4.4.2. In vitro trypanocidal assays

Monkey kidney cells (LLC-MK2 strain-ATCC) were resuspended in RPMI medium without phenol red (Gibco-BRL Life Technologies, Grand Island, NY) containing 10% fetal bovine serum (Life Technologies Inc., Bethesda, MD) and antibiotics (Sigma Chemical Co., St. Louis) at 2×10^3 cells/well and were cultured in 96-well plates for 24 h. The cells were infected with 1×10^4 trypomastigotes forms of *T. cruzi* Tulahuen strain, steadily expressing the β-galactosidase gene from *Escherichia coli*, and after 24 h compounds **9–13** were added at the indicated concentrations (0.5 mM–3.5 μM). After 4 days of culture, 50 μL of PBS containing 0.5% of Triton X-100 and 100 μM Chlorophenol Red-β-D-galactoside (CPRG-Sigma) were added. Plates were incubated at 37 °C for 4 h and absorbance was read at 570 nm.[56]

4.4.2.1. Cytotoxicity assay. Mammalian cell cytotoxicity was evaluated as previously.[60] Spleen cells from C57BL/6 mice were isolated by dissociation and incubated for 5 min with red blood cell lysis buffer (one part of 0.17 M Tris–HCl [pH 7.5] and nine parts of 0.16 M ammonium chloride). The cells were suspended in RPMI 1640 medium supplemented with 10% fetal bovine serum and cultured in flat-bottom 96-well plates at 5×10^5 cells/well with different concentrations of the compounds at 37 °C for 24 h. Tween 20 at 0.5% was used as cell death positive control. Cells were harvested, incubated with 10 μg/mL propidium iodide (Sigma) and acquired using a FACSCantoII (Becton-Dickinson Immunocytometry System Inc., San Jose, CA, USA). Data analysis was performed using FlowJo software (Ashland, Oregon, USA).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.11.022.

References and notes

1. Previato, J. O.; Andrade, A. F.; Pessolani, M. C. V.; Mendonca-Previato, L. *Mol. Biochem. Parasitol.* **1985**, *16*, 85.
2. Zingales, B.; Carniol, C.; de Lederkremer, R. M.; Colli, W. *Mol. Biochem. Parasitol.* **1987**, *26*, 135.
3. Cross, G. A. M.; Takle, G. B. *Annu. Rev. Microbiol.* **1993**, *47*, 385.
4. Schenkman, S.; Jiang, M. S.; Hart, G. W.; Nussenzweig, V. *Cell* **1991**, *65*, 1117.
5. de Lederkremer, R. M.; Agusti, R. *Adv. Carbohydr. Chem. Biochem.* **2009**, *62*, 311.
6. Buscaglia, C. A.; Campo, V. A.; Frasch, A. C. C.; Di Noia, J. M. *Nat. Rev. Microbiol.* **2006**, *4*, 229.
7. Mucci, J.; Risso, M. G.; Leguizamon, M. S.; Frasch, A. C. C.; Campetella, O. *Cell Microbiol.* **2006**, *8*, 1086.
8. Buschiazzo, A.; Amaya, M. F.; Cremona, M. L.; Frasch, A. C.; Alzari, P. M. *Mol. Cell* **2002**, *10*, 757.
9. Previato, L. M.; Previato, J. O.; Jones, C.; Xavier, M. T.; Parodi, A. J.; Wait, R.; Travassos, L. R. *J. Biol. Chem.* **1995**, *270*, 7241.
10. Vandekerckhove, F.; Schenkman, S.; de Carvalho, L. P.; Tomlinson, S.; Kiso, M.; Yoshida, M.; Hasegawa, A.; Nussenzweig, V. *Glycobiology* **1992**, *2*, 541.
11. Kim, J. H.; Won Ryu, H.; Shim, J. H.; Park, K. H.; Withers, S. G. *ChemBioChem* **2009**, *10*, 2475.
12. Arioka, S.; Sakagami, M.; Uematsu, R.; Yamaguchi, H.; Togame, H.; Takemoto, H.; Hinou, H.; Nishimura, Shin-Ichiro *Bioorg. Med. Chem.* **2010**, *18*, 1633.
13. Harrison, J. A.; Kartha, K. P.; Fournier, E. J. L.; Lowary, T. L.; Malet, C.; Nilsson, U. J.; Hindsgaul, O.; Schenkman, S.; Naismith, J. H.; Field, R. A. *Org. Biomol. Chem.* **2011**, *9*, 1653.
14. Meinke, S.; Schroven, A.; Thiem, J. *Org. Biomol. Chem.* **2011**, *9*, 4487.
15. Bradley, S. J.; Fazli, A.; Kiefel, M. J.; von Itzstein, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1587.
16. Kiefel, M. J.; von Itzstein, M. *Chem. Rev.* **2002**, *102*, 471.
17. Walz, G.; Aruffo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132.
18. Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833.
19. Titz, A.; Patton, J.; Smiesko, M.; Radic, Z.; Schwardt, O.; Magnani, J. L.; Ernst, B. *Bioorg. Med. Chem.* **2010**, *18*, 19.
20. Kuribayashi, T.; Ohkawa, N.; Satoh, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3307.
21. Jakab, Z.; Fekete, A.; Borbás, Anikó; Lipták, A.; Antus, S. *Tetrahedron* **2010**, *66*, 2404.
22. Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. *J. Org. Chem.* **1998**, *63*, 1074.
23. Gervay, J.; Flaherty, T. M.; Nguyen, C. *Tetrahedron Lett.* **1997**, *38*, 1493.
24. Von Itzstein, M. V. *Nat. Rev. Drug Disc.* **2007**, *12*, 967.
25. Von Itzstein, M. V.; Wu, W-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, L. M.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418.
26. Liakatos, A.; Kiefel, M. J.; Fleming, F.; Coulson, B.; Itzstein, M. V. *Bioorg. Med. Chem.* **2006**, *14*, 739.
27. Weiwer, M.; Chen, C.-C.; Kemp, M. M.; Linhardt, R. J. *Eur. J. Org. Chem.* **2009**, *16*, 2611.
28. Li, J.; Zheng, M.; Tang, W.; He, P.-L.; Zhu, W.; Li, T.; Zuo, J.-P.; Liu, H.; Jiang, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5009.
29. Aragão-Leoneti, V.; Campo, V. L.; Gomes, S. A.; Field, R. A.; Carvalho, I. *Tetrahedron* **2010**, *66*, 9475.
30. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
31. Purcell, W. P.; Singer, J. A. *J. Phys. Chem.* **1967**, *71*, 4316.
32. Nissink, J. W. M.; Mung, C.; Hartshorn, M.; Verdonk, M. L.; Cole, J. C.; Taylor, R. *Proteins* **2002**, *49*, 457.
33. Moris-Varas, F.; Qian, X.-H.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 7647.
34. Kovacs, J.; Pinter, I.; Szego, F.; Toth, G.; Messmer, A. *Acta Chim. Acad. Sci. Hung.* **1970**, *101*, 7.
35. Jeanloz, R. W.; Stoffyn, P. J. *Methods Carbohydr. Chem.* **1962**, *1*, 221.
36. (a) Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1994**, *251*, 33; (b) Danac, R.; Ball, L.; Gurr, S. J.; Fairbanks, A. J. *Carbohydr. Res.* **2008**, *343*, 1012.
37. Wang, H.; She, J.; Zhang, L.-H.; Ye, X.-S. *J. Org. Chem.* **2004**, *69*, 5774.
38. Öberg, C. T.; Noresson, A.-L.; Delaine, T.; Larumbe, A.; Tejler, J.; von Wachenfeldt, H.; Nilsson, Ulf J. *Carbohydr. Res.* **2009**, *344*, 1282.
39. Kumar, A.; Doddi, V. R.; Vankar, Y. D. *J. Org. Chem.* **2008**, *73*, 5993.
40. Poirot, E. *Carbohydr. Res.* **2001**, *334*, 195.

41. Nyffeler, P. T.; Liang, C.; Koeller, K. M.; Wong, C. *J. Am. Chem. Soc.* **2002**, *124*, 10773.
42. Lundquist, J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781.
43. Gan, Z.; Roy, R. *Can. J. Chem.* **2002**, *80*, 908.
44. Mereyala, H. B.; Gurrala, S. R.; Mohan, S. K. *Tetrahedron* **1999**, *55*, 11331.
45. Lacerda, V.; Silva, G. V. J.; Constantino, M. G.; Tormena, C. F.; Williamson, R. T.; Marquez, B. L. *Magn. Reson. Chem.* **2006**, *44*, 95.
46. Malapelle, A.; Coslovi, A.; Doisneau, G.; Beau, J.-M. *Eur. J. Org. Chem.* **2007**, *111*, 3145.
47. Gigue're, D.; Patnam, R.; Bellefleur, M.-A.; St.-, C.; Satob, S.; Roy, R. *Chem. Commun.* **2006**, 2379.
48. Heidecke, C. D.; Lindhorst, T. K. *Synthesis* **2006**, *1*, 161.
49. Hodosi, G.; Kovac, P. *Carbohydr. Res.* **1997**, *303*, 239.
50. Grindley, T. B. *Adv. Carbohydr. Chem. Biochem.* **1998**, *53*, 17.
51. Carvalho, I.; Andrade, P.; Campo, V. L.; Guedes, P. M. M.; Sesti-Costa, R.; Silva, J. S.; Schenkman, S.; Dedola, S.; Hill, L.; Rejzek, M.; Nepogodiev, S. A.; Field, R. A. *Bioorg. Med. Chem.* **2010**, *18*, 2412.
52. Campo, V. L.; Carvalho, I.; Silva, C. H. T. P.; Schenkman, S.; Hill, L.; Nepogodiev, S. A.; Field, R. A. *Chem. Sci.* **2010**, *1*, 510.
53. Neres, J.; Buschiazzo, A.; Alzari, P. M.; Walsh, L.; Douglas, K. T. *Anal. Biochem.* **2006**, *357*, 302.
54. Neres, J.; Brewer, M. L.; Ratier, L.; Botti, H.; Buschiazzo, A.; Edwards, P. N.; Mortenson, P. N.; Charlton, M. H.; Alzari, P. M.; Frasch, A. C.; Bryce, R. A.; Douglas, K. T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 589.
55. (a) Silva, R. S. F.; Costa, E. M.; Trindade, U. L. T.; Teixeira, D. V.; Pinto, M. D. F. R.; Santos, G. L.; Malta, V. R. S.; De Simone, C. A.; Pinto, A. V.; de Castro, S. L. *Eur. J. Med. Chem.* **2006**, *41*, 526; (b) Guedes, P. M.; Oliveira, F. S.; Gutierrez, F. R.; da Silva, G. K.; Rodrigues, G. J.; Bendhack, L. M.; Franco, D. W.; Do Valle Matta, M. A.; Zamboni, D. S.; da Silva, R. S.; Silva, J. S. *Br. J. Pharmacol.* **2010**, *160*, 270.
56. Buckner, F. S.; Verlinde, C. L.; La Flamme, A. C.; Van Voorhis, W. C. *Antimicrob. Agents Chemother.* **1996**, *40*, 2592.
57. Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon: New York, NY, 1980.
58. Schenkman, S.; Chaves, L. B.; Pontes de Carvalho, L. C.; Eichinger, D. *J. Biol. Chem.* **1994**, *269*, 7970.
59. Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 1286.
60. Silva, J. J. N.; Pavanelli, W. R.; Gutierrez, F. R. S.; Lima, F. C. A.; Silva, A. B. F.; Silva, J. S.; Franco, D. W. *J. Med. Chem.* **2008**, *51*, 4104.